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Antioxidant Metabolism in Cotton Seedlings Exposed to Temperature Stress in the Field

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ABSTRACT

Early season temperature stress adversely affects the growth and development of cotton (*Gossypium hirsutum* L.) seedlings. Oxidative damage resulting from temperature extremes is thought to be a cause of diminished seedling performance. Cotton (cv Fibermax 958) was planted at Lubbock, TX, in 2003 and 2004 to investigate the effect of low and high temperatures on oxidative stress and antioxidant metabolism in seedlings exposed to normal thermal variation. Early and late plantings in 2003 provided seedlings of different ages for comparisons. Malondialdehyde was slightly increased in response to low temperatures indicating some oxidative damage in the seedlings. The activities of ascorbate peroxidase and glutathione reductase were not altered in response to low or high temperatures. The glutathione pool was predominately reduced in all plantings in both years indicating sufficient reduced glutathione. It is concluded that the indicators of antioxidant metabolism varied in the seedlings but not in response to temperature variation. It is proposed that antioxidant metabolism in the seedlings was sufficient to mitigate oxidative damage with only minor alterations.

Environmental stress adversely affects plant performance and often results in significant reductions in crop yield and quality worldwide (Boyer, 1982). The exposure of plants to high and low air temperatures can result in the production of reactive oxygen species (ROS) that contribute to diminished plant performance (Grill et al., 2001, p. 262; Noctor and Foyer, 1998). Oxidative stress is a term commonly used to describe the adverse effects of ROS on plants. A variety of enzymatic and nonenzymatic mechanisms exist to metabolize ROS into less harmful chemical species. Recent reviews by Foyer (2001) and Blokhina et al. (2003) summarize much of the current knowledge of antioxidant metabolism in plants. However, field-level antioxidant studies have been limited with most studies focusing on acute exposures to water deficits, chemicals, and temperature stresses to produce oxidative damage.

Mahan and Wanjura (2005) performed field studies to identify changes in antioxidant metabolism in cotton subjected to season-long water deficits in the field. The results of that study indicated that glutathione metabolism changed significantly over the growing season but not in response to water deficits. Ascorbate peroxidase (APX) was found to increase under water stress in a manner that suggested a protective response. The amount of malondialdehyde (MDA), a compound correlated with oxidative damage in plants, did not increase in response to water deficits and it was proposed that oxidative damage was substantially mitigated in the plants under water stress without large changes in antioxidant metabolism. The absence of a water deficit response was attributed to the sufficiency of antioxidant protection in cotton under water stress in the field, particularly in light of the gradual nature of the development and alleviation of water deficits under field conditions. Simply stated, the plant had adequate time and resources to respond to changing water deficits.

Unlike water deficits, temperature stresses in the field can develop and be alleviated within hours, providing the plant less time to respond adaptively. Perhaps this is why effects of temperature stress on seedlings are generally recognized to have substantial negative effects on many crops. Agronomic considerations often require that crops be planted when temperatures are not optimal, and thus, in practical terms, exposure of seedlings to stressful temperatures is often unavoidable. This is particularly true in the case of cotton grown on the southern High Plains of Texas where high altitude and a relatively short growing season result in planting when temperatures are often below optimum. The objective of this study was to determine the effect, if any, of temperature variation on oxidative stress in cotton seedlings in the field. The hypothesis of the study is that rapid changes in environmental temperature will result in an increase in the oxidative by-product malondialdehyde and/or changes in components of antioxidant metabolism that are responsible for stress resistance. In an effort to address the hypothesis, cotton was planted on the southern High Plains of Texas in May in 2003 and 2004 to monitor antioxidant metabolism under natural thermal variation. An early and late planting date in 2003 provided a comparison of antioxidant response to temperatures in seedlings of different ages.

MATERIALS AND METHODS

To characterize changes in temperature associated with the passage of weather fronts during the seedling interval, temperature was monitored at 2 m above the plant canopy with a shielded thermocouple. Plant temperatures near the soil surface are sufficiently correlated with those measured at 2 m to detect weather-related temperature changes that may affect seedling antioxidant metabolism (Rosenberg et al., 1983, p. 127). Temperature was measured every 6 s and 15-min averages were recorded with a data logger.

Plant Materials

Cotton (*G. hirsutum* cv. Fibermax 958) was planted on 23 April (DOY 113) in 2003 and 13 May (DOY 133) in both

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Abbreviations: APX, ascorbate peroxidase; DOY, day of year; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; ROS, reactive oxygen species.

2003 and 2004. The first plant samples were collected on DOY 140 in 2003 and on DOY 145 in 2004. In 2003, sampling began 20 and 3 d after emergence for the early and late planting dates respectively and 5 d after emergence in 2004.

Plants were sampled from DOY 140 to 164 in 2003 and DOY 145 to 175 in 2004. Plants in 2003 were sampled over an interval from 20 to 40 and 3 to 27 d after emergence respectively for early and late plantings. Plants in 2004 were sampled 5 to 35 d after emergence.

In an effort to observe oxidative damage and/or antioxidant changes extant at the beginning of the plant's daily photosynthetic period, plant samples for metabolic analyses were collected between 0800 and 0830 h. Samples were collected early in the day to minimize any diurnal differences in antioxidant metabolism that were not related to temperature. Samples consisted of cotyledons until a primary leaf of 4 cm^2 was present, after which, the uppermost leaf of 4 cm^2 was collected. While cotyledons and leaves differ in several respects, young cotyledons and leaves are both photosynthetic sources and thus oxidative damage to either may negatively affect seedling growth and development. At each sampling date, a minimum of three samples were collected with each sample consisting of material from a minimum of three plants. All samples were transported to the laboratory within minutes of collection, frozen in liquid nitrogen, and stored at −90°C until analysis.

Spearman Rank Correlation Sampling

Since plant samples were collected between 0800 and 0830 h, the daily maximum temperature occurred after the plant sample for that day was collected. Any metabolic responses to high temperature on a given day would be evidenced in plant samples from the following day. In the Spearman rank correlation procedure, the correlations with low temperature used minimum temperature and metabolic indicator data from the same day, while correlations with high temperatures compared the high temperature on a given day with the metabolic indicator data from the following day. This methodology does not allow for the detection of any effects that might occur between the occurrence of the maximum temperature for a day and the plant sampling on the following morning.

In an effort to determine if there was a delay between temperature variation and a metabolic response, rank correlations were determined on a variable time lag in which temperature for a given day was compared with the ranks of metabolic indicators for subsequent days up to 3 d following the temperature event. These lagged correlations varied though not in a manner that altered the significance of the outcomes. Therefore all rank correlations are reported for comparisons of minimum temperature and metabolic indicators on the same date and maximum temperature and metabolic indicators with a 1-d adjustment as described above.

Glutathione Reductase Assay

The assay for glutathione reductase (GR) was modified from Gossett et al. (1994). GR was extracted from frozen cotton material by grinding the tissue in a mortar and pestle to a powder in liquid nitrogen and 25% (w/w) polyvinylpolypyrrolidone (PVPP). The powder was then ground with ice cold extraction buffer consisting of 0.1 m*M* ethylenediaminetetraacetic acid (EDTA), 0.2% (v/v) Triton X100, 2.0% (w/w) polyvinylpyrrolidone (PVP), 10 m*M* isoascorbate (added before extracting tissue) in 0.1 *M* Tris-HCl buffer pH 7.0 at a ratio of 0.4 g to 3 mL. The extract was centrifuged for 30 min at 15 000 × *g* at 4°C. The supernatant was used in the assays. A 1.0-mL assay contained 0.5 m*M* oxidized glutathione (GSSG) and 0.15 m*M* reduced nicotinamide adenine dinucleotide phosphate (NADPH) in 0.05 *M* Tris-HCl pH 7.5. The assay was initiated with the addition of 0.05 mL leaf extract. If the activity in the extract was greater than 0.03 units the extract was diluted with buffer (0.05 *M* Tris-HCl, pH 7.5) and assayed again. Enzyme activity was monitored by the decrease in absorbance at 340 nm at 25°C for 30s and the initial rate determined. A unit of activity is the amount of enzyme that will catalyze the reduction of 1.0 μmol of GSSG per min at 25°C.

Glutathione Measurements

The amount and form of glutathione were determined by a modification of the method of Gossett et al. (1994) which measured total glutathione (reduced glutathione (GSH) and GSSG) by a glutathione reductase catalyzed reaction. Removal of GSH from the sample by chemical derivatization provided for the quantification of GSSG in the sample. Frozen tissue (0.33 g) was homogenized on ice with a tissue homogenizer in 6% *m*-phosphoric acid (pH 2.8) and 1.0 m*M* EDTA to reduce the oxidation of GSH. The extract was centrifuged at 15 000 × *g* for 1 h at 4°C and the diluted extract used in subsequent assays. The extract was diluted 1:30 in 5% sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$), pH 7.5, immediately before use. The 0.9-mL volume assay contained 0.3 mL Reagent A, 0.24 mL Reagent B, 0.3 mL diluted extract and 0.06 mL 0.009 *M* NADPH to initiate the reaction. Reagent A consisted of 0.11 *M* sodium phosphate monobasic heptahydrate ($NaH_2PO_4 \cdot 7H_2O$), 0.04 *M* $NaH_2PO_4 \cdot H_2O$, 0.015 *M* EDTA, 0.3 m*M* 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), and 0.04% bovine serum albumin (BSA). Reagent B contained 0.001 *M* EDTA, 0.05 *M* Imidazole, 0.02% BSA, and 1.5 units/mL GR. The assay mixture (minus the NADPH) was allowed to equilibrate at 25°C for 2 min. The increase in absorbance at 412 nm was monitored for 30 s after the addition of the NADPH. A reaction blank was prepared with 5% $NaH_2PO_4 \cdot H_2O$, pH 7.5, in place of the plant extract. The concentration of glutathione was determined through comparison with a standard curve of reaction rate as a function of GSSG (0.05–0.4 μg/0.9 mL in 5% $NaH_2PO_4 \cdot H_2O$, pH 7.5). Glutathione values are expressed as GSH equivalents (1.0 mole GSSG is equivalent to 2.0 moles of GSH). Each reported value represents the mean of a minimum of three determinations. The amount of oxidized glutathione in the extract was determined by removing GSH by derivatization with 2-vinylpyridine. The derivatizations were performed immediately after leaf extractions to minimize the oxidation of GSH in the sample. Diluted plant extract (2.0 mL) and 0.08 mL 2-vinylpyridine were added to a culture tube and the solution was mixed vigorously. The derivatizations were performed at 25°C for 1 h and the resultant solution was used as plant extract for the glutathione assay previously described.

Ascorbate Peroxidase Assay

The extraction and assay procedures for APX are described in Allen (1995). Cotton material (0.1 g) was ground to a powder in a mortar and pestle using liquid nitrogen. The powder was homogenized in 1.0-mL extraction buffer with a tissue homogenizer on ice. The extraction buffer consisted of 1.0 m*M* ascorbate, 1.0 m*M* EDTA, 1% (v/v) Triton X100, and 20% (v/v) glycerol in 50 m*M* HEPES buffer, pH 7.0, prepared immediately before the extraction. The extract was centrifuged (15 000 × *g*) for 30 min at 4°C. The supernatant fraction was used for the assays. All reagents and the extract were prepared fresh before the assay. The assay reaction mixture contained 1.0 m*M* EDTA, 1.0 m*M* hydrogen peroxide, 0.5 m*M*

ascorbate, and 30 μL of the extract in 50 m*M* HEPES buffer, pH 7.0, in a total volume 1.0 mL. The assay was allowed to equilibrate at 25°C for 1 min before the addition of hydrogen peroxide, which initiated the reaction. The reaction was monitored as a decrease in absorbance at 290 nm for 1.0 min at 25°C and the initial rate determined. A control reaction was prepared by replacing the ascorbate with the HEPES buffer. A unit of ascorbate peroxidase is defined as the amount necessary to oxidize 1.0 μmol of ascorbate/min at 25°C (290 nm extinction coefficient of 2.8 mm^{-1} cm^{-1}).

Malondialdehyde Assay

The lipid peroxidation assays were performed according to a modification of the method of Hodges et al. (1999), which corrects for the presence of other compounds that absorb at 532 nm. Frozen cotton tissue (0.24 g) was ground in liquid nitrogen until powdery and extracted in 6 mL 80% ethanol. The extract was centrifuged (15 000 × *g*) for 30 min and the supernatant used for the assays. The supernatant (0.5 mL) was reacted with 20% trichloroacetic acid, 0.01% butylated hydroxytoluene, and 0.65% thiobarbituric acid at 95°C for 25 min in a total volume of 1.5 mL. A control reaction consisted of the reaction mixture substituting water for the thiobarbituric acid. The assay mixture was cooled to room temperature and centrifuged (15 000 × *g*) for 10 min. The absorbance of each sample was determined at 400 nm, 532 nm and 600 nm. The concentration of MDA was calculated using the following equations as developed by Hodges et al. (1999).

$$A = [(\text{Abs } 532_{+\text{TBA}}) - (\text{Abs } 600_{+\text{TBA}}) - (\text{Abs } 532_{-\text{TBA}} - \text{Abs } 600_{-\text{TBA}})]$$

$$B = [(\text{Abs } 440_{+\text{TBA}} - \text{Abs } 600_{+\text{TBA}}) \times 0.0571]$$

$$\text{MDA equivalents (nmol/mL)} = (A - B/157\,000) \times 10^6$$

RESULTS AND DISCUSSION

Temperature

Studies of the effect of temperature on antioxidant metabolism often utilize temperature regimes that include some combination of what can be characterized as below optimal, optimal, and above optimal temperatures. These regimes are often imposed as various day/night temperature values. Rivero et al. (2004) reported the effect of elevated temperature on antioxidants in tomato on the basis of comparisons of plants grown at 25°C and 35°C for 30 d. Jiang and Huang (2001b) monitored antioxidant activity of turf grasses grown under optimal (20°C day/15°C night) and high (35°C day/30°C night) temperature regimes. While such studies have provided information on the effect of stable temperatures on antioxidants, cotton seedlings on the southern High Plains of Texas are frequently subject to diurnal as well as weekly temperature variation that may have effects not observed in studies such as those previously noted.

To make comparisons between temperature measured in this study and historic temperature records, the temperatures in this study were measured at a height of 2 m above the soil surface. Though the temperature near the soil surface is different from that measured at 2 m, they are correlated and the weather variation experienced near the soil surface is correlated with that at a height of 2 m. Figure 1 shows the pattern of air temperatures over the interval from DOY 138 through 175 during 2003 (a) and 2004 (b). The minimum and maximum temperatures in 2003 were 10 and 38°C. Minimum and maximum temperatures for 2004 were 11 and 33°C. The mean temperatures for the experimental interval were 22°C in 2003 and 25°C in 2004.

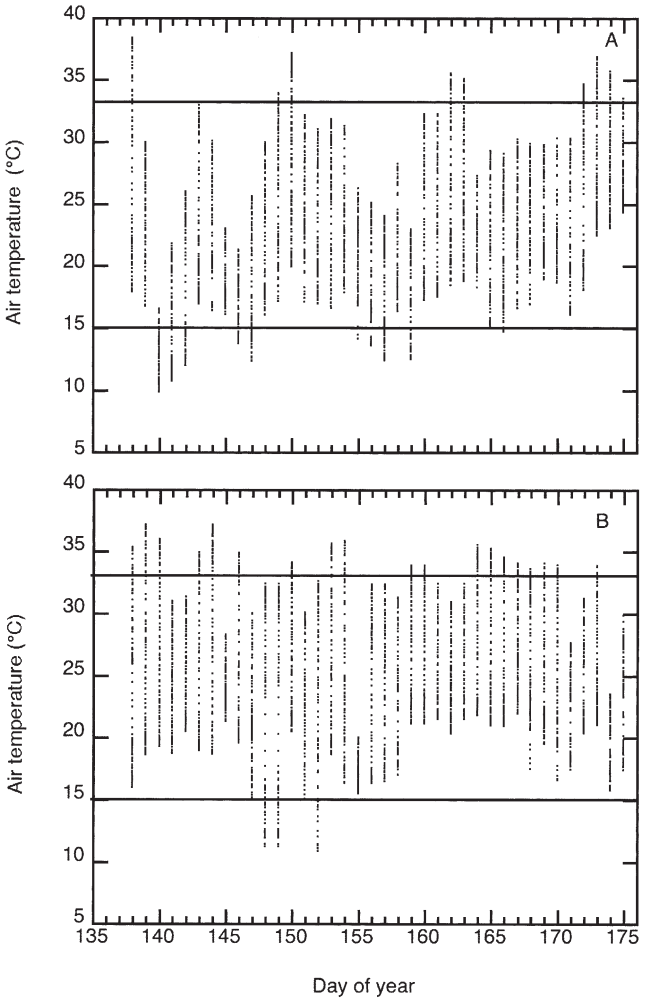

Fig. 1. Air temperatures measured over the interval 14 May to 23 June 2003 (a) and 2004 (b) at Lubbock, TX. Average temperature for 15-min intervals was measured with a thermocouple. Horizontal lines at 15°C and 33°C indicate thresholds for low and high temperature stresses.

For the purposes of this study, temperatures ≤15°C were considered to represent stressful low temperatures and those ≥33°C were considered as potentially stressful high temperatures. The definition of low temperature stress is based on a general consensus that temperatures ≤15°C are stressful for cotton seedlings and result in diminished seedling performance (Gipson, 1986). High temperature stress was defined as temperatures ≥33°C on the basis of the work of Burke et al. (1985) that showed the induction of a "heat shock" response in cotton at that temperature and the fact that seedling temperatures can be elevated over air temperatures due to their proximity to the soil surface. The data indicate that 2003 was the cooler of the years with 11 d with tempera-

tures ≥33°C and 11 d with temperatures ≤15°C. In 2004, there were 19 d with stressful high temperatures (≥33°C) and only 5 d with stressful low temperatures (≤15°C).

The effects of temperature on the plant are in some respects instantaneous and continuously changing. The temporal relationship between a given temperature and its affect on the plant in potentially complex and an almost infinite number of comparisons are possible between the occurrence of a given temperature and a metabolic response in the plant. This study focused on responses appearing as a change in antioxidant metabolism or oxidative damage that would accumulate over the course of several days, indicating an adaptive response (e.g., changes in enzyme activity) or cumulative damage (e.g., increased malondialdehyde levels).

Abrupt Temperature Transitions

One characteristic of early season temperature stress that may be particularly problematic is short-duration episodes of relatively rapid transitions from high to low temperature followed by a return to more moderate temperatures. In an effort to document the frequency of abrupt temperature transitions, a temperature change from a high temperature ≥33°C followed within 48 h by a low temperature ≤15°C was defined. The analysis of the temperatures during the experimental interval indicates that there were two abrupt temperature transition events in both 2003 (DOY 138–140, 163–165) and 2004 (DOY 146–148, 150–152). An analysis of 54 yr of weather records from the southern High Plains of Texas (Crosbyton, TX) indicated that at least one abrupt temperature transition occurred in 61% of the years with 35% of the years having two or more abrupt temperature transitions. These results demonstrate that the thermal variation events observed in this study are representative of the environment experienced by cotton in the field on the southern High Plains of Texas and the potential importance of such temperature events on cotton seedlings.

Antioxidants

Malondialdehyde

MDA is an indicator of oxidative-stress related peroxidation of membrane lipids and its concentration has been shown to correlate with peroxides resulting from temperature and water stress related membrane damage (Halliwell and Gutteridge, 1989). Huang et al. (2001) reported increased MDA content in response to high soil temperatures in two cultivars of creeping bentgrass (*Agrostis palustris* Huds.) that were grown in sand-filled tubes for 42 d. While MDA was elevated by stress in both heat sensitive and tolerant cultivars, it was higher in the heat sensitive cultivar.

The amount of malondialdehyde varied (7.7- and 4.7-fold) in the early and late planting of 2003 respectively over the interval with minimum and maximum values of 11.0 and 49.0 nmol gfw^{-1} (mean = 26 nmol gfw^{-1}) for the older plants and minimum and maximum values of 15.0 and 113 nmol gfw^{-1} (mean = 38 nmol gfw^{-1}) for the younger plants (Fig. 2). While the minimum values were similar, the maximum and mean values were higher for the older plants. In 2004 MDA varied (2.7-fold) over the interval with minimum and maximum values of 16 to 44 nmol gfw^{-1} (mean = 33 nmol gfw^{-1}). MDA levels in the early planting of 2003 were elevated at early sampling dates while levels in the late plantings in both years were more stable over the entire sampling interval. The relationship between low temperature and the amount of MDA was investigated by a Spearman rank correlation (Wilks, 1995, p. 50) comparing minimum daily temperature and MDA amount (Table 1). The rankings of MDA and minimum temperature indicate that malondialdehyde levels were negatively and significantly correlated with low temperatures over the measurement interval for early ($r = -0.54$) and late ($r = -0.56$) plantings in 2003 and positively correlated ($r = 0.15$) in 2004. This suggests that there was increased oxidative damage to the plants at the lower temperatures in 2003 with less damage in 2004. The relationship between high temperature and the amount of MDA was investigated by a Spearman rank correlation comparing maximum daily temperature and MDA amount (Table 1). The rankings of MDA and maximum temperature indicate no significant correlation in the late ($r = -0.26$) or early ($r = -0.06$) plantings in 2003. There was a nonsignificant positive correlation (0.55) in 2004. Malondialdehyde did not increase at high temperatures to the same extent seen at low temperatures.

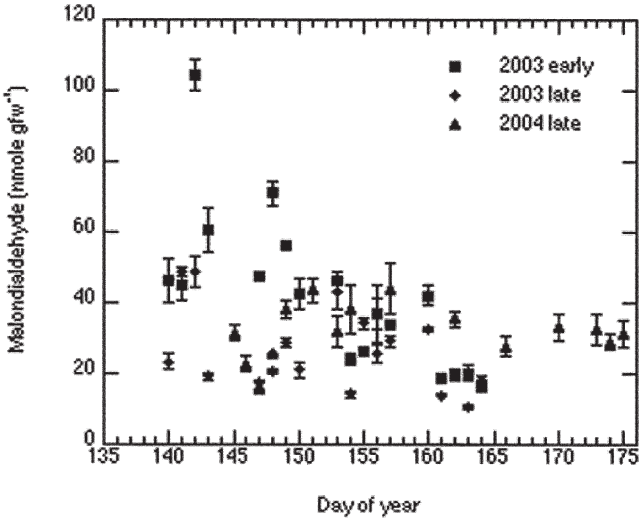

Fig. 2. Levels of malondialdehyde (nmol g^{-1} fresh wt.) in cotton tissues from early planting (DOY 113) in 2003 and late plantings (DOY 133) in 2003 and 2004 at Lubbock, TX. Each data point represents a minimum of 3 samples. Error bars indicate standard error of the mean.

The correlation between MDA amount and daily minimum temperature suggests that there was some degree of low temperature oxidative damage. The failure to see similar increases with higher temperatures suggests less high temperature oxidative damage occurred.

Glutathione Pool

Two changes in glutathione metabolism were anticipated in response to temperature stress in this study:

Table 1. Correlation between temperature and oxidative stress indicators. A Spearman rank correlation analysis was performed with daily minimum and maximum temperatures and oxidative stress indicators. Correlation coefficients are shown for rank correlations of temperature with malondialdehyde (MDA), ascorbate peroxidase activity (APX), glutathione reductase activity (GR), total glutathione (GLUT), oxidized glutathione (GSSG), and the ratio of GSH to GSSG (GSH/GSSG).

		Oxidant indicator					
Temperature	Planting	MDA	APX	GR	GLUT	GSSG	GSH/GSSG
Minimum	Early 2003	−0.54*	0.45	−0.04	nd	nd	nd
	Late 2003	−0.56*	−0.05	−0.34	−0.06	−0.02	0.38
	Late 2004	0.15	−0.06	−0.22	0.08	−0.15	0.62
Maximum	Early 2003	−0.06	0.30	−0.16	nd	nd	nd
	Late 2003	−0.26	−0.50*	−0.46*	0.02	0.09	0.23
	Late 2004	0.55	0.01	−0.19	−0.33	−0.03	−0.56

* Denotes significance at a 95% confidence level.
nd, Not determined.

increased glutathione content and/or an increase in the fraction of the glutathione pool in the oxidized form. Glutathione was measured only in the late plantings of 2003 and 2004. Figure 3 shows the pattern of the amount and form of glutathione in 2003 (a) and 2004 (b). The total glutathione varied over the sampling interval 3.9-fold in 2003 and 2.7-fold in 2004. Mean values of total glutathione were 448 and 299 μg gfw^{-1} in 2003 and 2004, respectively. The amount of GSSG varied 3.5-fold in 2003 and 3.2-fold in 2004. The mean of GSSG was 132 μg gfw^{-1} in 2003 and 148 μg gfw^{-1} in 2004. The amount of GSH varied 5.2-fold in 2003 and 4.5 in 2004. In 2003, the mean value of GSH was 316 and 150 μg gfw^{-1} in 2004. With only two exceptions in 2003 and four in 2004, the glutathione pool was predominately reduced with no evidence of a sustained trend toward oxidation.

The relationship between low temperature and the amount and form of glutathione was investigated by a Spearman rank correlation comparing minimum daily temperature and glutathione (Table 1). The rankings of total glutathione and minimum temperature indicate that total glutathione levels were uncorrelated with lower temperatures over the measurement interval for the late planting ($r = -0.06$) in 2003 and in 2004 ($r = 0.08$). The nonsignificant correlations between maximum temperature and total glutathione were $r = 0.02$ in 2003 and $r = -0.33$ in 2004. GSSG was uncorrelated with minimum temperature ($r = 0.02$) and maximum temperature ($r = 0.09$) in 2003. In 2004 GSSG was not correlated with minimum temperature ($r = -0.15$) or maximum temperature ($r = -0.03$).

The ratio of reduced to oxidized glutathione (GSH/GSSG) was not significantly correlated with minimum temperature in 2003 ($r = 0.038$) or 2004 ($r = 0.62$). The GSH/GSSG ratio was not significantly correlated with maximum temperature in 2003 ($r = -0.56$) or 2004 (0.23).

The variation in amount and form of glutathione was not significantly correlated with either high or low temperatures suggesting a sufficiency of glutathione amount and form. In general, the majority of glutathione in an unstressed plant cell is found in the reduced form, GSH, typically comprising 70 to 90% of the total (Bielawski and Joy, 1986; Smith, 1985). Under oxidative stress, if the rate of glutathione oxidation exceeds the rate of glutathione reduction, an increase in the fraction of glutathione in the oxidized form, GSSG, can result (Sgherri and Navari-Izzo, 1995; Smith, 1985). Sgherri and Navari-Izzo (1995) reported changes in the glutathione metabolism of sunflowers (*Helianthus annuus* L.) under water stress in the field. Changes in glutathione content and form resulting from oxidative stresses were identified in experiments involving glutathione metabolism in catalase deficient barley (*Hordeum vulgare* L.) by Smith et al. (1985) who reported increases in the total glutathione content and oxidized glutathione in H_2O_2 stressed barley plants. Increased glutathione content has been reported to be related to a feedback inhibition of glutathione synthesis by GSH (Richman and Meister, 1975; Hell and Bergmann, 1990; Schneider and Bergmann, 1995), and the depletion of reduced glutathione has been postulated to initiate increased glutathione synthesis under oxidative stress. Since there was no temperature-related increase in oxidized glutathione in this study, an increase in total glutathione pool would not be anticipated.

Glutathione Reductase Activity

The enzyme GR plays an important role in antioxidant metabolism by maintaining the glutathione pool in a reduced state and thus the maintenance of a pool of GSH is important for the plant. The pattern of the activity of GR in the seedlings is shown in Fig. 4. GR activity in 2003 varied (4.0- and 9.6-fold) in both the early and late plantings respectively over the interval with minimum and maximum values of 0.27 and 1.10 units gfw^{-1} (mean = 0.67 units gfw^{-1}) for the older plants and minimum and maximum values of 0.16 and 1.55 units gfw^{-1} (mean = 0.67 units gfw^{-1}) for the younger plants. In 2004, GR activity varied (1.5-fold) with minimum and maximum values of 0.48 and 0.74 units gfw^{-1} (mean = 0.58 units gfw^{-1}). The activity of GR was elevated at the initial sampling dates in both plantings in 2003 and declined by the middle of the sampling interval. Activity in 2004 was relatively stable across the sampling interval.

The effect of low temperature on GR activity was investigated by a Spearman ranking correlation comparing minimum daily temperature and GR activity (Table 1). The rankings of GR and minimum temperature in 2003 indicate nonsignificant correlations ($r = -0.34$, increased GR activity) in the younger plants and older plants ($r = -0.04$). In 2004 there was a nonsignificant negative correlation ($r = -0.22$). This suggests that GR activity was at best slightly affected by low temperatures. The effect of high temperature on GR activity was inves-

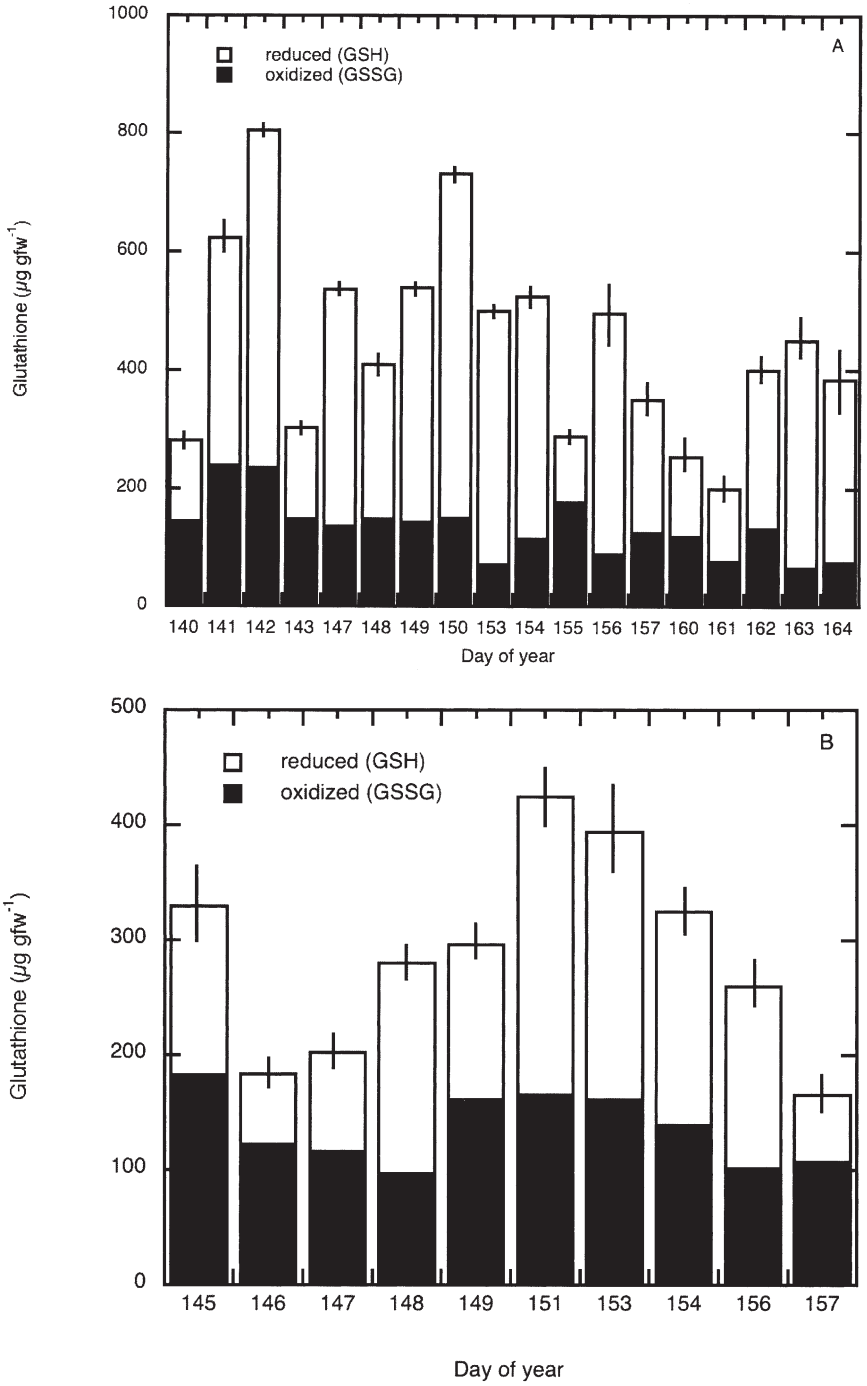

Fig. 3. Levels of glutathione (μg gfw^{-1}) in cotton tissues from late plantings (DOY 133) in 2003 (a) and 2004 (b) at Lubbock, TX. Bar increments indicate amount of oxidized and reduced glutathione and the total pool size is represented by the aggregate of the two. Each data point represents a minimum of three samples. Error bars indicate standard error of the mean.

tigated by a Spearman rank correlation comparing maximum daily temperature and GR activity (Table 1). In 2003, the rankings of GR and maximum temperature indicate nonsignificant negative correlations in the younger plants ($r = -0.46$) and older plants ($r = -0.16$). In 2004 a nonsignificant negative correlation ($r = -0.19$) with high temperature was observed.

GR has been reported to vary in response to environmental stresses. Foster and Hess (1980) found that GR was increased in cotton leaves exposed to an oxygen-enriched atmosphere. Stress-related changes in both the amount and form of glutathione, as well as the activity of GR, were reported by Sgherri and Navari-Izzo (1995). They found increases in glutathione amount and GR activity in the leaves of sunflower following exposure to water stresses in the field. When exposed to severe water deficits they found that the glutathione pool became more oxidized and both the total glutathione pool and activity of GR declined. In another study that involved stress exposures under field conditions, Gam-

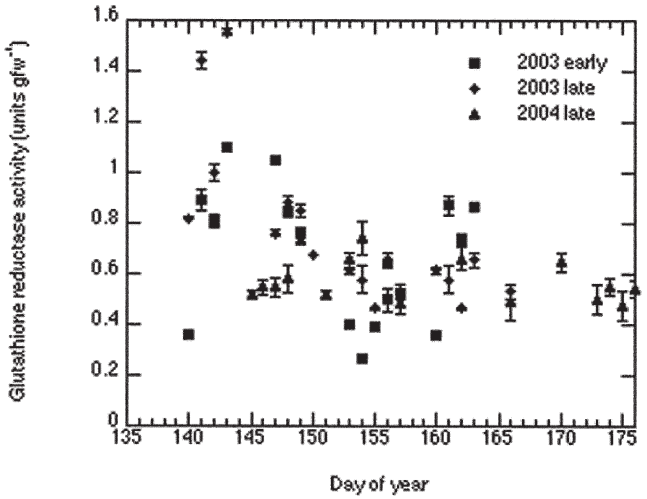

Fig. 4. Activity of glutathione reductase (units gfw^{-1}) in cotton tissues from early planting (DOY 113) in 2003 and late plantings (DOY 133) in 2003 and 2004 at Lubbock, TX. A unit of activity is the amount of enzyme that will catalyze the reduction of 1 μmol of GSSG per min at 25°C. Each data point represents a minimum of three samples. Error bars indicate standard error of the mean.

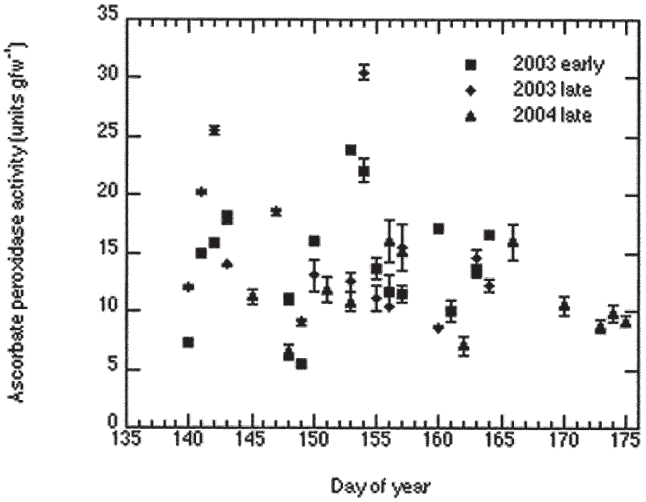

Fig. 5. Activity of ascorbate peroxidase (units gfw^{-1}) in cotton tissues from early planting (DOY 113) in 2003 and late plantings (DOY 133) in 2003 and 2004 at Lubbock, TX. A unit of ascorbate peroxidase is defined as the amount necessary to oxidize 1 μmole of ascorbate per minute at 25°C (290-nm extinction coeffcient = 2.8 mM^{-1} cm^{-1}). Each data point represents a minimum of three samples. Error bars indicate standard error of the mean.

ble and Burke (1984) monitored the activity of GR in the leaves of wheat (*Triticum aestivum* L.) grown under different levels of water and temperature stress. They reported that GR activity was increased under water deficits in what they suggested was an adaptive response.

Ascorbate Peroxidase Activity

The pattern of the activity of APX in the seedlings is shown in Fig. 5. APX activity in 2003 varied (5.0- and 5.3-fold) in both the younger and older plants, respectively, over the interval with minimum and maximum values of 6.12 and 30.57 units gfw^{-1} (mean = 15.10 units gfw^{-1}) for the late planting, and minimum and maximum values of 5.43 and 28.70 units gfw^{-1} (mean = 15.12 units gfw^{-1}) for the early planting. While the magnitude of variation and the mean values were similar for the late and early plantings the pattern of the variation differed. In 2004 APX activity varied (2.5-fold) with minimum and maximum values of 6.5 and 16 units gfw^{-1} (mean = 11.1 units gfw^{-1}).

The relationship between low temperature and APX activity was investigated by a Spearman rank correlation comparing minimum daily temperature and APX activity (Table 1). The rankings of APX and minimum temperature were not significantly correlated in the younger plants ($r = -0.05$) or in the older plants ($r = 0.45$). This suggests that APX activity was unaffected or decreased at lower temperatures.

The relationship between high temperature and APX activity was investigated by a Spearman rank correlation comparing maximum daily temperature and APX activity (Table 1). The rankings of APX and maximum temperature were not significantly correlated in the younger plants ($r = -0.50$) or older plants ($r = 0.30$). In this study, APX activity was not significantly correlated with either minimum or maximum temperature.

CONCLUSIONS

The goal of this study was to document the response of indicators of antioxidant metabolism in cotton seedlings to temperature variation. On the southern High Plains of Texas low temperature stress is generally recognized as a potential problem for cotton seedlings with the effect of elevated temperatures generally considered less important. Cotton seedlings grown in 2003 and 2004 experienced stress periods of temperatures ≤15°C and periods of temperatures ≥33°C. On a gross level, there were no apparent indications of temperature damage to the seedlings during or following the measurement period. The crop in both years reached maturity and produced yields that were representative for the region. Regardless of the end of season status of the plants, there is still the possibility that there was oxidative stress that to some degree adversely affected the seedlings and their subsequent growth and development. In other words, what might have been the final yield? The amount of MDA (an indicator of oxidative damage) in the seedlings was correlated negatively with minimum temperature over the experimental interval. This finding suggests that oxidative damage resulted from the exposure of the seedlings to low temperatures. The 26 nmol gfw^{-1} mean amount of MDA measured in this study is similar to levels reported in unstressed samples by Massardo et al. (2000) for oats (*Avena sativa* L.) (15–20 nmol gfw^{-1}), Wu and Wang (2003) for corn (*Zea mays* L.) (30 nmol gfw^{-1}) and somewhat higher than those reported in mature cotton leaves (10 nmol gfw^{-1}) by Mahan and Wanjura (2005). The amount of MDA varied by 7.7- and 4.7-fold in the early and late plantings of 2003 and 2.7-fold in 2004. The levels MDA and the extent of variation suggest stress-related increases in lipid peroxidation. Jiang and Huang (2001a) reported a twofold increase in MDA in leaves of two cool season grasses in response

to high temperature stress. Similarly, Huang et al. (2001) reported 3- and 5-fold increases in MDA in response to high temperature stress in cultivars of creeping bentgrass. Munro et al. (2004) reported that MDA levels in etiolated mung bean (*Vigna radiata* L.) seedlings were not altered by low temperatures.

In this study, MDA levels were negatively correlated with minimum temperature, suggesting that low temperatures did result in oxidative damage in the form of lipid peroxidation. The correlation between MDA level and maximum temperature was smaller than that with low temperature perhaps indicative of less oxidative damage. While the increases in MDA suggest that there was oxidative stress, the lack of increases in the activity of enzymes that are known to increase in plants under oxidative stress may be evidence that the stress was mitigated by the normal levels of antioxidant metabolism. Alternatively, the oxidative stress may not have been sufficient to produce an enhancement of antioxidant metabolism. The seasonal sufficiency of antioxidant metabolism in cotton under water stress was proposed by Mahan and Wanjura (2005) in a study of water stress effects on antioxidants in field grown cotton. They reported that antioxidant metabolism varied significantly on a seasonal basis but not in response to water deficits. Given that there was a capacity for increased antioxidant metabolism but no evidence of such an increase under stress, they proposed that antioxidant metabolism may be sufficient to protect the plant from the oxidative stresses associated with the temperatures experienced by the plants in this study. In light of the relative constancy of at least some degree of oxidative stress in plants, even under optimal temperature conditions, there would be a strong advantage for the plant to have antioxidant activity in excess of the level needed for protection under normal conditions. Additional evidence for the sufficiency of antioxidant metabolism may be found in the work of Payton et al. (2001) who genetically manipulated cotton to increase the activities of glutathione–ascorbate cycle enzymes in the chloroplast and observed increased resistance to chilling-related photooxidative stress in the laboratory. Subsequent field trials (Logan et al., 2003) did not exhibit enhanced chilling tolerance. The value of a baseline level of oxidative stress protection in the seedling is apparent in light of the pattern of temperature variation observed in this study. Cotton seedlings on the southern High Plains of Texas are routinely exposed to relatively large changes in temperature over periods of less than 2 d. If the plant were to alter antioxidant metabolism in response to such changes, it is possible that by the time the metabolism had responded to a low temperature stress, the temperatures may have returned to a nonstressful level or even risen to the point that the plant was experiencing high temperature stress. In a highly variable environment, it might be most advantageous to have sufficient antioxidant metabolism to protect against the normal thermal variation experienced by the plant. The findings of the present study perhaps help to explain why some efforts to alter antioxidant metabolism have successfully enhanced the resistance to oxidative stress in the laboratory but failed to improve plant performance in the field.

This study focused on the response of oxidative stress indicators in cotton seedlings to low and high temperatures. It was hypothesized that temperature-related oxidative stress would result in some combination of the following: increased MDA content, increased activities of APX and GR, and alterations in the amount and form of glutathione. Analysis of the data revealed correlations between temperature and the oxidative stress indicators. While some of the correlations were statistically significant, the magnitude of the observed responses, when compared with relevant literature, suggests that plants did not experience a detrimental level of oxidative stress and/or a physiologically important response in antioxidant metabolism. It is concluded that antioxidant metabolism in the cotton seedlings of this study was sufficient to ameliorate the temperature-related oxidative stresses in this study.

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